31. An isolated protein disulfide isomerase polypeptide that has a sequence identity of at least 85% based on the Clustal method when compared to an amino acid sequence selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, and 20.

32. The polypeptide of Claim 31 wherein the sequence identity is at least 90%.

33. The polypeptide of Claim 31 wherein the sequence identity is at least 95%.

34. The polypeptide of Claim 31 wherein the polypeptide has a sequence selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18, and 20.

35. An isolated polynucleotide that (1) comprises at least 300 contiguous nucleotides and (2) remains hybridized with the isolated polynucleotide of Claim 16 under a wash condition of 0.1X SSC, 0.1% SDS, and 65°C.

REMARKS

AMENDMENTS

Applicants respectfully submit that the amendments to the Specification only correct obvious typographical and clerical errors.

Applicants submit that the newly added claims more clearly and distinctly recite that which applicants consider to be their invention, and are adequately supported by the original disclosure. For example, support for 90% and 95% is found on page 9 at lines 9 and 11, respectively.

No new matter is believed to be at issue. Entry of the amendments and early favorable consideration of the claims on the merits are hereby respectfully requested.

RESTRICTION REQUIREMENT

Examiner asserted that in the former claims there were three distinct inventions:

- Group I (Claims 1-9, 14, and 15), drawn to compositions and methods comprising nucleic acids which encode polypeptides.
- Group II (Claim 10), drawn to polypeptides.
- Group III (Claims 11-13), drawn to methods of selecting polynucleotides that affect levels of polypeptide expression.

Applicants have canceled all former claims, without prejudice or disclaimer and have written new Claims 16-35. The subject matter recited in the alleged Group I is now encompassed by new Claims 16-30 and 35. The subject matter recited in alleged Group II is now encompassed by new Claims 30-34. The subject matter recited in alleged Group III is no longer claimed.

In response to the Restriction Requirement, Applicants elect, with traverse, the subject matter of alleged Group I. Applicants further elect, with traverse, the corn protein disulfide isomerase of SEQ ID NO:10 encoded for by the nucleotide sequence in SEQ ID NO:9.

Applicants traverse the restriction requirement as follows.

Restriction Between a Polynucleotide and a Polypeptide Encoded by the Polynucleotide

Applicants traverse the restriction between alleged Groups I and II. Claims in alleged Group I recite nucleotide molecules that encode polypeptides recited in claims of Group II.

For two claims to be properly restricted, they must be (1) independent *and* distinct, and (2) pose a serious burden on the Examiner. See MPEP §803. Applicants respectfully assert that a claim to a protein is neither independent from, nor distinct of another claim to the polynucleotide encoding the protein. Therefore, even under the curious interpretation by the PTO that the phrase "independent and distinct" in 35 U.S.C. §121 means "independent or distinct," a protein claim is NOT restrictable from its corresponding DNA claim.

According to the MPEP, the term "independent inventions" has the same meaning as "unrelated inventions", and

> [t]he term "independent" (i.e. not dependent) means that there is no disclosed relationship between the two or more disclosed subjects disclosed, that is, they are unconnected in design, operation, or effect . . .

MPEP §802.01.

Examples of dependent subjects used in the same section of the MPEP include (1) process and apparatus used in the practice of the process; (2) composition and the process in which the composition is used; and (3) process and the product made by such process.

There is absolutely no argument that a protein molecule and the DNA molecule encodes it are unrelated. Therefore, the Examiner clearly is not basing his restriction requirement on the argument that the two type of claims are independent. By requiring restriction between one claim to a *particular* gene sequence and another claim to the polypeptide encoded by the gene, the Examiner essentially asserts that claims in these two groups are "distinct."

According to the MPEP, "[t]he term "distinct" means that two or more subjects are . . . PATENTABLE (novel and unobvious) OVER EACH OTHER (though they may each be patentable over the prior art)." MPEP §802.01 (*capitalization original*).

In other words, the Examiner is asserting that the polypeptide is patentable over the specific gene molecule that encodes the polypeptide. Applicants, however, respectfully disagree, and submit that the nucleic acid molecule and the protein it encodes are not "distinct" from each other as the term is used in the context for restriction requirement analyses.

One only needs rudimentary training in molecular biology to recognize that, when a particular nucleic acid molecule is known, there is only one protein that corresponds to this *particular* DNA molecule. See *In re Deuel*, 34 USPQ2d 1210, 1211 (Fed. Cir. 1995)[1] ("[O]nce a cDNA's nucleotide sequence is known, the amino acid sequence of the protein it codes may be predicted using the genetic code. . .") In other words, with the assistance of the Genetic Code, the nucleotide sequence of the gene can be translated into the corresponding amino acid sequence of the protein with the "particularity and precision" required by the law such that the protein is anticipated by the disclosed gene sequence. Even if one argues a single reference disclosing the gene sequence is not sufficiently anticipatory, the specific polypeptide at least obvious over the gene sequence. See *In re Deuel*, 34 USPQ2d at 1215 ("A prior art disclosure of a process *reciting a particular compound* or an obvious variant thereof as a product of the process . . . rais[es] issues of anticipation under 35 U.S.C. §102 as well obviousness under §103." (*emphasis original*)).

Despite codon degeneracy, the court in *Deuel* indicated that the complete *amino acid sequence* of the protein *may render obvious* a broad claim reciting all *DNA sequences* coding for the protein. *In re Deuel*, 34 USPQ2d at 1215. It is thus clear from *In re Deuel* that when a reference that predicts the precise structural characteristics of a compound, this compound is both anticipated and obvious in view of this reference. See also *In re Petering*, 301 F.2d 676 (CCPA 1962); *In re Brown*, 141 USPQ 245, 329 F.2d 1006, (CCPA 1964).

Because it is simply implausible to assert that a protein is novel and unobvious over the DNA molecule that encodes it, a claim drawn to a protein is not distinct from a claim that is drawn to a DNA that encodes the same protein.[2]

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[1] In *Deuel*, the PTO rejected Deuel's claims to specific nucleic acid molecules as obvious in view of a reference that discloses the partial amino acid sequence of the enzyme (human and bovine HBGFs) encoded by the gene, in combination with a reference that teaches the general cloning methodology. The Court of Appeals for the Federal Circuit reversed, holding that, (1) due to codon degeneracy, the cited references neither disclose nor suggest the *precise* claimed nucleic acid molecules, and (2) partial amino acid sequence of a protein does not render a broad claim to all nucleic acid molecules that encode the full length protein obvious, *In re Deuel*, 34 USPQ2d at 1215.

[2] Applicants further note that the Examiner has not made a distinction between a claim to a polynucleotide that encodes a polypeptide without any

Furthermore, applicants' position is consistent with authorities under the PCT, to which the United States is a party, and prevailing practices throughout the world. According to Example 17, Part 2, Annex B, of the PCT Administrative Instructions, unity of invention exists between Protein X and a DNA sequence encoding Protein X.

Consequently, applicants respectfully submit that a restriction requirement between claims in alleged Groups I and II is improper, and these claims should be rejoined and examined together on the merits.

Sequence Election Requirement

Applicants further respectfully traverse the restriction to only one of the disclosed nucleic acid or amino acid sequences. Examiner asserts that each DNA and protein represented by a SEQ ID NO is a distinct product and must be elected in addition to the Group.

However, the Examiner has ignored published agency policy that permits a reasonable number of nucleic acid and amino acid sequences to be examined in one application without restriction. According to the MPEP,

> the Commissioner has decided *sua sponte* to partially waive the requirements of 37 CFR 1.141 et seq. and permit a reasonable number of such nucleotide sequences to be claimed in a single application. See Examination of Patent Applications Containing Nucleotide Sequences, 1192 O.G. 68 (November 19, 1996).
>
> It has been determined that normally ten sequences constitute a reasonable number for examination purposes. Accordingly, in most cases, up to ten independent and distinct nucleotide sequences will be examined in a single application without restriction. In addition to the specifically selected sequences, those sequences which are patentably indistinct from the selected sequences will also be examined. Furthermore, nucleotide sequences encoding the same protein are not considered to be independent and distinct inventions and will continue to be examined together.

MPEP §803.04.

Under this policy, in most cases, up to 10 independent and distinct nucleotide sequences ***will*** be examined in a single application without restriction. Those sequences which are patentably indistint from the sequences selected by the applicant will also be examined, and nucleotide sequences encoding the same protein are ***not*** considered to be independent and distinct inventions and will continue to be examined together.

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specified function, because the Examiner did not indicate former dependent Claim 2, which recites the encoded polypeptide to be the specifically disclosed, known to be functional, sequence, e.g., SEQ ID NO:2.

Applicants respectfully submit that the Examiner is bound by the rules and regulations or other public notices contained in the MPEP. Accordingly, applicants submit that this one-sequence-only requirement is improper and should be withdrawn.

New claims 16-35 written to the instant application refer to 10 nucleotide sequences and 10 polypeptides encoded by said nucleotide sequences. These 10 polynucleotides encode protein disulfide isomerases or fragments thereof.

Because not more than 10 ***independent and distinct*** sequences are claimed, all the sequences should be examined together.

Conclusion

In view of the amendments and remarks above, applicants respectfully submit that the restriction requirements are improper and should be withdrawn. Applicants earnestly await a favorable Office Action on the merits. The Examiner is welcome to contact the undersigned if there is any question.

Respectfully submitted,

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Dated: April 12, 2001

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In showing the changes, deleted material is shown in bolded brackets and stricken through, and inserted material is shown underlined.

Paragraph starting at page 4, line 12:

It is preferred that the isolated polynucleotides of the claimed invention consist of a nucleic acid sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, and 19 that codes for the polypeptide selected from the group consisting of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16, 18 and 20. The present invention also relates to an isolated polynucleotide comprising a nucleotide sequence**[**s**]** of at least **[**~~one of~~**]**40 (preferably at least **[**~~one of~~**]**30, most preferably at least **[**~~one of~~**]**15) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, and 19 and the complement of such nucleotide sequences.

Paragraph starting at page 5, line 11:

The present invention relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of a protein disulfide isomerase precursor or an RB60 polypeptide**[** ~~gene~~**]**, preferably a plant protein disulfide isomerase precursor or an RB60 polypeptide**[**~~gene~~**]**, comprising the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least **[**~~one of~~**]**40 (preferably at least **[**~~one of~~**]**30, most preferably at least **[**~~one of~~**]**15) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, and 19 and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a portion of a protein disulfide isomerase precursor or an RB60 amino acid sequence.

Paragraph starting at page 6, line 11:

In the context of this disclosure, a number of terms shall be utilized. As used herein, a "polynucleotide" is a nucleotide sequence such as a nucleic acid fragment. A polynucleotide may be a polymer of RNA or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA, or synthetic DNA. An isolated polynucleotide of the present invention may include at least **[**~~one of~~**]**40 contiguous nucleotides, preferably at least **[**~~one of~~**]**30 contiguous nucleotides, most preferably **[**~~one of~~**]**at least 15 contiguous nucleotides, of the nucleic acid sequence of the SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, or 19.

Paragraph starting at Page 7, line 21:

Substantially similar nucleic acid fragments may be selected by screening nucleic acid fragments representing subfragments or modifications of the nucleic acid fragments of the instant invention, wherein one or more nucleotides are substituted, deleted and/or inserted, for their ability to affect the level of the polypeptide encoded by the unmodified nucleic acid fragment in a plant or plant cell. For example, a substantially similar nucleic acid fragment representing at least **[~~one of~~]**30 contiguous nucleotides derived from the instant nucleic acid fragment can be constructed and introduced into a plant or plant cell. The level of the polypeptide encoded by the unmodified nucleic acid fragment present in a plant or plant cell exposed to the substantially similar nucleic fragment can then be compared to the level of the polypeptide in a plant or plant cell that is not exposed to the substantially similar nucleic acid fragment.

Paragraph starting on page 7, line 32:

For example, it is well known in the art that antisense suppression and co-suppression of gene expression may be accomplished using nucleic acid fragments representing less than the entire coding region of a gene, and by nucleic acid fragments that do not share 100% sequence identity with the gene to be suppressed. Moreover, alterations in a nucleic acid fragment which result in the production of a chemically equivalent amino acid at a given site, but do not effect the functional properties of the encoded polypeptide, are well known in the art. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a functionally equivalent product. Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the polypeptide molecule would also not be expected to alter the activity of the polypeptide. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products. Consequently, an isolated polynucleotide comprising a nucleotide sequence of at least **[~~one of~~]**40 (preferably at least **[~~one of~~]**30, most preferably at least **[~~one of~~]**15) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and the complement of such nucleotide sequences may be used in methods of selecting an isolated polynucleotide that affects the expression of a polypeptide (such as PDI precursor or PDI RB60) in a host cell. A method of selecting an isolated polynucleotide that affects the level of expression of a polypeptide in a host cell (eukaryotic, such as plant, or prokarotic such as yeast bacterial or virus) may comprise the steps of: constructing an isolated polynucleotide of the present

invention or an isolated chimeric gene of the present invention; introducing the isolated polynucleotide or the isolated chimeric gene into a host cell; measuring the level a polypeptide in the host cell containing the isolated polynucleotide; and comparing the level of a polypeptide in the host cell containing the isolated polynucleotide with the level of a polypeptide in a host cell that does not contain the isolated polynucleotide.

Paragraph starting at page 10, line 6:

"Codon degeneracy" refers to divergence in the genetic code permitting variation of the nucleotide sequence without ~~[effecting]~~affecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment comprising a nucleotide sequence that encodes all or a substantial portion of the amino acid sequences set forth herein. The skilled artisan is well aware of the "codon-bias" exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a nucleic acid fragment for improved expression in a host cell, it is desirable to design the nucleic acid fragment such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

Paragraph starting at page 14, line 7:

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA 85*:8998-9002) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA 86*:5673-5677; Loh et al. (1989) *Science 243*:217-220). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques 1*:165). Consequently, a polynucleotide comprising a nucleotide sequence of at least ~~[one of]~~40 (preferably ~~[one of]~~at least 30, most preferably ~~[one of]~~at least 15) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and the complement of such nucleotide sequences may be used in such methods to obtain a nucleic acid fragment encoding a

substantial portion of an amino acid sequence of a polypeptide (such as PDI precursor or PDI RB 60). The present invention relates to a method of obtaining a nucleic acid fragment encoding a substantial portion of a polypeptide [~~of a gene~~](such as PDI precursor or PDI RB 60) preferably a substantial portion of a polypeptide of a plant gene, comprising the steps of: synthesizing an oligonucleotide primer comprising a nucleotide sequence of at least [~~one of~~]40 (preferably at least [~~one of~~]30, most preferably at least [~~one of~~]15) contiguous nucleotides derived from a nucleotide sequence selected from the group consisting of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and the complement of such nucleotide sequences; and amplifying a nucleic acid fragment (preferably a cDNA inserted in a cloning vector) using the oligonucleotide primer. The amplified nucleic acid fragment preferably will encode a portion of a polypeptide (such as PDI precursor or PDI RB 60).